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The influence of H/D kinetic isotope effect on radiation-induced transformations of hydroxyl-containing compounds in aqueous solutions

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Abstract

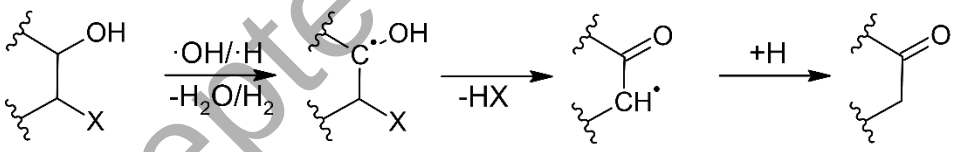
Vicinal diols and its derivatives can be exploited as model compounds for the investigation of radiation-induced free-radical transformations of hydroxyl-containing biomolecules such as carbohydrates, phospholipids, ribonucleotides, amino acids and peptides. In this paper, for the first time, the prospects of isotope reinforcement approach in inhibiting free-radical transformations of hydroxyl-containing compounds in aqueous solutions are investigated on the example of radiolysis of 1,2-propanediol and 1,2-propanediol-2-d_1 aqueous solutions. At an absorbed dose rate of 0.110±0.003 $Gy \cdot s^{-1}$ a profound kinetic isotope effect (KIE) is observed for the non-branched chain formation of acetone, which is a final dehydration product of predominant carbon-centered radicals $CH_3 \cdot C(OH)CH_2OH$. In 0.1 and 1 M deaerated solutions at pH 7.00±0.01, the values of KIE are 8.9±1.7 and 15.3±3.1, respectively. A rationale for the fact that a strong KIE takes place only in the case of chain processes, which may occur during free-radical transformations of vicinal diols, is also provided herein based on the results of 2-propanol and 2-propanol-2-d_1 indirect radiolysis. Lastly, the lack of KIE is shown in the case of 2-butanone formation from 2,3-butanediol or 2,3-butanediol-2,3-d_2. This indicates that the type (primary, secondary) of the β-carbonyl radical formed as a result of $CH_3 \cdot C(OH)CH(OH)R$ (R = H, CH_3) dehydration determines the manifestation of the effect.

Keywords: 1,2-propanediol, 2,3-butanediol, 2-propanol, steady-state radiolysis, deuterium, kinetic isotope effect.

Introduction

Classical radiobiology interprets the direct ionization of a cell nucleus and particularly nuclear DNA as the main process of the cell damage arising from exposure to ionizing radiation (IR) [1]. However, with the development of the field and the accumulation of experimental data, it was shown that indirect damage by cellular water radiolysis products can make a much greater contribution to the modification of biomolecules [2–4]. This is especially true for biological membranes [5], which can be considered the second most important localization of IR-induced cell defects. The major components of a mammalian cell membrane are lipids (phospho-, glyco-, sphingo-, cholesterol), and it also contains carbohydrates and proteins in integrated or linked forms [6]. In the presence of oxygen, with which the lipid bilayer is enriched in comparison to the surrounding aquatic medium [7,8], chain lipid peroxidation (LPO) occurs, affecting polyunsaturated fatty acid (PUFA) moieties. Though there are a plethora of other free-radical degradation and modification processes affecting biomolecules [9], LPO is arguably the most studied one [10].

However, in different tissues, the oxygen level varies in a wide range [11]. At low (hypoxic) oxygen levels, the role of LPO reduces significantly, which provokes other pathways of free-radical damage to biomolecules. Our research group develops the idea that in addition to well-characterized LPO, free-radical fragmentation processes also occur with varying probability in biological systems exposed to IR. This fragmentation is characteristic of compounds containing a free hydroxyl group adjacent to a hydroxyl/ester/amino/amide group [12]:



(1)

X = OH, OR, OC(O)R, NH_2, NHR, NHC(O)R, OPO_3^{2-}, etc.

It is reliably established that physiologically active carbonyl and HX products of the process (1) are formed in various systems of glycerophospholipids and lysophospholipids [13–20], sphingolipids [13,18,20], carbohydrates [21,22] and glycolipids [23,24], amino acids and their derivatives [25–27]. The accumulation of fragmentation products is particularly noticeable in hypoxic irradiation conditions since O_2 is known to inhibit the elimination of HX in the process (1). Substances, such as quinones and phenolic compounds, capable of forming quinoid structures [28–32], B group vitamins [33], and nitroazoles [31,32], also suppress the process (1) due to oxidation of intermediate α-hydroxyalkyl radicals or by scavenging the initiators (mainly

·OH). Nevertheless, the development of new ways to regulate the probability of the process (1) remains pertinent.

Relatively recently, a fundamentally different approach to suppression of free-radical reactions has emerged, the concept of which consists of increasing the resistance of substrates themselves, based on KIE[1]. It was shown [34–37] that PUFAs reinforced site-specifically with deuterium in the bis-allylic methylene groups (namely in there) are profoundly protected from LPO due to the KIE arising from the rate-limiting propagation step.

It was uncovered that a linoleic acid sample enriched with linoleic acid-11,11-d_2 by a fifth is profoundly resistant to the chain oxidation as a whole. The observed "20% effect" means that the therapeutic effect of the isotope reinforcement can be reached practically *in vivo* by proper dietary supplementation [34]. Studies in yeast and mammalian myoblast models, as well as atherosclerosis and Alzheimer's disease models in mice, have shown that feeding living cells/oral supplementation with deuterated PUFAs (D-PUFAs) is safe and reduces pro-oxidant and iron-induced injury [34,36,37]. Moreover, the use of ethyl linoleate-11,11-d_2 (known as RT001) in clinical trials was approved by the United States Food and Drug Administration agency (FDA), and some results have been obtained. Thus, the beneficial effect of RT001 has been recognized in patients with neurodegenerative conditions known to involve LPO, such as Friedreich Ataxia and Infantile Neuroaxonal Dystrophy [38,39].

The aforementioned LPO studies have shown that the isotope reinforcement approach is the promising modality for the protection of biomolecules from free-radical degradation. Thus, the objective of the present work is to find out how the H/D substitution in the α-C position to the OH group affects the fragmentation of hydroxyl-containing compounds (Scheme (1)) induced by γ-rays. Here we mainly focus on the protiated (ordinary) and deuterated forms of 1,2-propanediol and 2,3-butanediol, which are prone to dehydration, and 2-propanol, which, in contrast, possesses simpler radiation chemistry. This research provides a quantitative comparison of the processes taking place during the steady-state indirect radiolysis of these compounds in deaerated aqueous solutions.

[1] KIE describes rate change due to isotopic substitution at a site of bond breaking/formation (primary KIE) or another site (secondary KIE) in the rate-determining step (RDS) of a mechanism. The KIE value is defined as the ratio of rate constants belonging to the reaction with the unsubstituted and substituted reactant. The RDS is usually slowed down in the case of H/D substitution.

Materials and methods

Chemicals

The substrates were 1,2-propanediol (≥ 99.5%, racemic mixture, Aldrich), 2-propanol (≥99.8%, Sigma-Aldrich) and 2,3-butanediol (≥98.0%, mixture of D-, L- and meso- isomers, Aldrich Chemistry) as well as their deuterated analogues. Monopotassium phosphate (≥ 99.0%, Sigma Life Sciences) and disodium phosphate (≥ 99.0%, Sigma-Aldrich) were used to prepare buffer solutions. Formaldehyde (37 wt. % solution in water containing 10-15% methanol as a stabilizer, Sigma-Aldrich), acetaldehyde (≥99.5%, Sigma-Aldrich), propanal (≥ 98.0%, Supelco Analytical), acetone (≥99.9%, AppliChem), hydroxyacetone (≥95%, Fluka Chemika), 2-butanone (≥99.0%, Sigma-Aldrich), were utilized as analytical standards in chromatographic assays. Methanol (MeOH) (AppliChem) and 2,4-dinitrophenylhydrazine (DNPH) (Sigma-Aldrich) were HPLC and reagent grade (97%), correspondingly, sulfuric acid – 96 wt. % solution in water (Acros Organics). Water was purified and deionized using a Siemens Ultra Clear TWF Water Purification System (France). Other chemicals used in this study were of the highest quality available.

Synthesis of deuterated substrates

Reagents from commercial suppliers were used without further purification. Diethyl ether (Et_2O) and tetrahydrofuran (THF) were dried over $LiAlH_4$. ^1H (500 MHz) and ^{13}C (126 MHz) NMR spectra were recorded with a Bruker DRX-500 spectrometer (Bruker, USA). Chemical shifts were referenced to the residual solvent signals of $CDCl_3$ (7.26 ppm for ^1H and 77.16 ppm for ^{13}C). Column chromatography was performed using 60 Å silica gel (40–63 μm). Analytical thin-layer chromatography was performed on Kieselgel 60 F254 precoated aluminium TLC plates (Merck).

2-Propanol-2-d_1

This compound was synthesized according to the published procedure [40]. $NaBD_4$ (2.0 g, 47.6 mol) was dissolved in NaOH aqueous solution (60 ml, 0.1 M). Acetone (14.0 ml, 18.9 mmol) was added dropwise to the solution of $NaBD_4$, keeping the solution temperature below 20^{o}C. The reaction mixture was additionally stirred at room temperature for 2 h, then sulfuric acid (5 M) was added until the pH of the solution reached a value of 1-2. A crude product was distilled off at 80-83^{o}C. The product was redistilled at 80-81^{o}C with the addition of a small amount of anhydrous calcium chloride. Thus, 2-propanol-2-d_1 (5.8 g, 50%) was obtained as a colorless liquid. Chemical purity (determined by the GC-MS method described in section 2.7): 95 wt. %

of 2-propanol-2-d_1, 0.06 wt. % of acetone. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.14 (s, 6H), 2.47 (br s, 1H) (Fig. S1 in *Supplemental materials*).

1,2-Propanediol-2-d_1

A solution of hydroxyacetone (0.74 g, 10 mmol) in THF (5 ml) was added dropwise for 10 min to a suspension of $LiAlD_4$ (0.54 g, 12.9 mmol) in THF (15 ml) cooled to –20 °C. The reaction mixture was slowly (30 min) warmed to room temperature, after which it was further stirred for 12 h. The reaction mixture was treated while cooling in an ice bath by sequential careful addition of water (0.6 ml), 15% NaOH solution (0.6 ml), and again water (1.7 ml), followed by stirring at room temperature for 2 h. The reaction mixture was filtered, the precipitate was additionally washed with THF (4 × 10 ml), after which the solvent was removed under reduced pressure to obtain 0.56 g of the relatively pure product. Additional purification was carried out by column chromatography on silica gel (eluent CH_2Cl_2/ethyl acetate (EtOAc)/hexane from 1:1:0.5 to 1:1:0, and then pure EtOAc), which led to 0.40 g (52%) of the colorless oily product. Chemical purity (determined by the LC-UV method described in section 2.5): 97 wt. % of 1,2-propanediol-2-d_1, ≤ 2 mol.% of hydroxyacetone. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.06 (s, 3H), 3.30 (d, 1H, J = 11.5 Hz), 3.50 (d, 1H, J = 11.5 Hz), 4.28 (br s, 2H) (Fig. S2 in *Supplemental materials*). ^{13}C NMR ($CDCl_3$, 126 MHz): δ 18.6 (s), 67.8 (m), 77.1 (t, J = 22 Hz) (Fig. S3 in *Supplemental materials*).

2,3-Butanediol-2,3-d_2 (mixture of diastereomers)

A solution of 2,3-butanedione (0.86 g, 10 mmol) in THF (5 ml) was added dropwise for 10 min to a suspension of $LiAlD_4$ (0.54 g, 12.9 mmol) in THF (15 ml) cooled to –20 °C. The reaction mixture was slowly (30 min) warmed to room temperature, after which it was further stirred for 12 h. The reaction mixture was treated while cooling in an ice bath by sequential careful addition of water (0.6 ml), 15% NaOH solution (0.6 ml), and again water (1.7 ml), followed by stirring at room temperature for 2 h. The reaction mixture was filtered, the precipitate was additionally washed with THF (4 × 10 ml), after which the solvent was removed under reduced pressure to obtain 0.68 g of the relatively pure product. Additional purification was carried out by column chromatography on silica gel (eluent CH_2Cl_2/EtOAc/hexane from 1:1:0.5 to 1:1:0, and then pure EtOAc), which led to 0.52 g (57%) of the product (colorless oil) as a mixture of diastereomers in a 6.5:1 ratio. ^{1}H NMR ($CDCl_3$, 500 MHz) of major diastereomer: δ 1.05 (s, 6H), 3.66 (br s, 2H) (Fig. S4 in *Supplemental materials*). ^{13}C NMR ($CDCl_3$, 126 MHz) of major diastereomer: δ 16.7, 70.4 (t, J = 21.6 Hz) (Fig. S5 in *Supplemental materials*).

Sample preparation

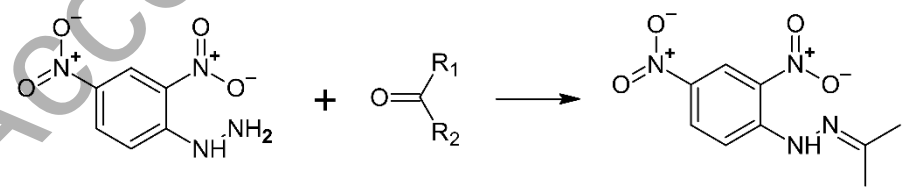
All solutions (including standard ones for chromatographic analysis) were prepared gravimetrically and necessary concentrations were obtained by serial dilution in 50 mM phosphate buffer (pH 7.00±0.01). The buffer was prepared by mixing 50 mM solutions of KH_2PO_4 and Na_2HPO_4 in deionized water under pH control using a Hanna HI 9321 pH-meter. Before each use, the instrument was calibrated using standard Hamilton Duracal buffers (Hamilton Bonaduz AG, Switzerland), which have pH values of 4.01±0.01 and 10.01±0.01. The derivatization reagent for HPLC analysis was prepared by dissolving 0.03 g DNPH in 6.2 mL 36 wt. % HCl solution followed by dilution up to 100 mL by MeOH.

Irradiation

Irradiation experiments were carried out once (1 M 1,2-propanediol-2-d_1), twice (1 M 1,2-propanediol, 0.01 and 0.1 M 1,2-propanediol-2-d_1), three (0.01 and 0.1 M 1,2-propanediol, 0.1 M 2-propanol, 0.1 M 2-propanol-2-d_1) or four (0.1 M 2,3-butanediol and 0.1 M 2,3-butanediol-2,3-d_2) times (irradiation on different days) with a ^{60}Co γ-radiation source MPX-γ25M (Belarus) at the adsorbed dose rate of 0.110±0.003 $Gy \cdot s^{-1}$, which had previously been established by the standard ferrous sulfate (Fricke) dosimetry ($G(Fe^{3+})$ = 1.62 $\mu mol \cdot J^{-1}$). Doses ranging from 0 (control) to 660 Gy were applied in increments of 132 Gy. The samples were irradiated under normal conditions for temperature and pressure (25°C and 1 atm). The radiolysis was performed in glass vessels (the sample volume was of 1.0 ml) sealed after being subjected to argon (≥99.993% purity) purge for 60 min to remove dissolved oxygen.

Analysis of irradiated 1,2-propanediol and 1,2-propanediol-2-d_1 solutions

Detection and quantification of carbonyl products were performed after the pre-column derivatization with DNPH (R_1, R_2 = H, Alk):



(2)

For this purpose, the derivatization reagent, preparation of which is described in the *Sample preparation* (section 2.3), was used.

The analysis was performed using a Shimadzu VP series LC system (Shimadzu, Japan), which includes the following integrated units: an LC-10AD VP pumping unit, an SCL-10A VP system controller, a CTO-10A column oven equipped with a manual injector, an SPD-10A VP UV–Vis

detector and a DGU-14A degasser. Chromatographic separation was carried out in a LiChrospher 100 RP-18e column (25 cm × 4.6 mm ID; 5 μm particle size, 100 Å pore size) in the isocratic mode of elution at 40^{o}C. The mobile phase consisted of MeOH and water (60:40, v/v) and the flow rate was equal to 1.0 mL·min^{-1}. The injection volume was 5 μL. The detection of hydrazone derivatives was performed at 366 nm.

A sample solution and the derivatization reagent were mixed in a 1:1 (v/v) ratio, incubated for 15 min and then 5 μL of the mixture was manually entered into the injector using a 25 μL Hamilton syringe (Hamilton Company, USA). Concentrations of the products were calculated using calibration curves obtained in the concentration range of 10-1000 μM for each substance.

Analysis of irradiated 2-propanol and 2-propanol-2-d_1 solutions

The accumulation of acetone in 2-propanol or 2-propanol-2-d_1 solutions was determined by gas chromatography using a Shimadzu GC-2010 (Japan) instrument equipped with an AOC-5000 auto-injector (Shimadzu, Japan) and a flame ionization detector (FID) (Shimadzu, Japan). Compounds were separated using a Supelcowax 10 (Supelco, USA) capillary column (30.0 m x 0.25 mm ID, 0.50 μm film thickness) with helium as a carrier gas in a constant flow rate of 1.28 mL·min^{-1}. The temperature of the injector port was set at 250^{o}C. The column temperature increased from 40^{o}C to 80^{o}C in increments of 5^{o}C·min^{-1}, then it rose to 210^{o}C at a rate of 8^{o}C·min^{-1} and finally was maintained at 210^{o}C for 5 min. The injection volume was 1 μL. The detector temperature was adjusted to 220^{o}C. The acetone concentrations were calculated using the calibration curve obtained in the concentration range of 10-1000 μM.

Analysis of irradiated 2,3-butanediol and 2,3-butanediol-2,3-d_2 solutions

The irradiated solutions were analyzed by GS-MS using a Shimadzu GC-2010 (Japan) instrument equipped with an AOC-20i auto-injector (Shimadzu, Japan) and fitted to a GCMS-QP2010 Plus quadrupole mass detector (Shimadzu, Japan). Compounds were separated using a Stabilwax-DA (Restek, USA) capillary column (30.0 m x 0.25 mm ID, 0.25 μm film thickness) with helium as a carrier gas in a constant flow rate of 1.02 mL·min^{-1}. The temperature of the injector port was set at 250^{o}C and the column temperature was programmed to increase from 40^{o}C to 150^{o}C in increments of 5^{o}C·min^{-1}. The injection volume was 1 μL. The MS operated in a positive electron impact (EI) mode with an electron energy of 70 eV (full scan, 40–200 m/z) and at the ion source temperature equal to 200^{o}C. Concentrations of the products were calculated using calibration curves obtained in the concentration range of 10-500 μM for each substance.

Use of chromatograms and statistical treatment of the results

In the case of symmetrically shaped peaks rising above a stable horizontal baseline, well separated from one another, concentration values were derived from both calculations using areas and heights of the peaks, otherwise, only peak areas were used to calculate concentrations. Preprocessing of chromatograms was performed in OriginPro software, Version 2019b (9.65), OriginLab Corporation, Northampton, MA, USA, https://www.originlab.com/.

Each G-value (and its standard error) was calculated as a best-fit value of the linear regression slope (and its standard error) of c(product) [μM] = f(D) [Gy]. Each linear regression analysis was performed on a dataset of 5 or 6 points (including 0 values) using the method of least squares. An error given for a specific G-value in *Results* (section 3) refers to the 95% confidence interval of the pooled mean calculated from the t-distribution using a set of G-values possessing the standard errors. To compare the radiation chemical yields of products for protiated and deuterated substrates, unpaired parametric t-tests were executed and two-tailed P values (95% confidence level) were reported. The null hypothesis is that two populations have the same mean (or that the isotopic substitution has no effect). A P-value, which is less than the threshold (α = 0.05) leads to the rejection of the null hypothesis and means that the difference is significant statistically. All the above-mentioned processing was performed using GraphPad Prism, version 8.0.1 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

Results

Effects of the H/D substitution in the position C_2 of 1,2-propanediol on its radiation-induced transformations in deaerated aqueous solutions

To assess the effect of deuterium introduction into the position C_2 of 1,2-propanediol, we performed radiolysis of 1,2-propanediol and 1,2-propanediol-2-d_1 deaerated solutions (pH = 7.0±0.1). Among six major compounds, which are formed from 1,2-propanediol during its indirect radiolysis [12], five products (hydroxyacetone, formaldehyde, acetaldehyde, acetone, and propanal) were detected using LC-UV assay following the pre-column derivatization with DNPH. Another product, 2-hydroxypropanal, has not been defined due to the lack of an analytical standard. Anyway, no clear peaks, which might belong to 2-hydroxypropanal, were observed in the full chromatograms recorded. Fig. 1 shows the difference between the ratio of carbonyl products formed from the protiated and deuterated substrates. Moreover, the chromatograms also demonstrate the effect of the substrate concentration on the amount of the substances produced.

<**Fig. 1**>

G-values of the carbonyl products related to different substrate concentrations are shown in Table 1.

<**Table 1**>

By definition, the value of the kinetic isotope effect is the ratio of rate constants of the corresponding reactions (k_H/k_D). In radiation-chemical experiments, the observed G-values of product accumulation/substrate decomposition are more often used, and under certain conditions, k_H/k_D is $G_H(X)/G_D(X)$ since the radiation-chemical yield is a kinetic parameter that describes the rate of product accumulation or substrate decomposition depending on the absorbed dose. Thus, in the case of stationary radiolysis of aqueous solutions of organic substances, this equality is observed when comparing G-values (except those of pure water radiolysis products, such as H_2) at the same substrate concentrations and experimental conditions [41]. Therefore, the value of KIE will be understood further as the ratio $G_H(X)/G_D(X)$ and can be found in the result tables after the corresponding P-value, if applicable.

Two main results follow from the analysis of Table 1 and Fig. 1. Firstly, the yields of products **4-6** do not depend significantly on the 1,2-propanediol concentration, whereas for product **1**, and

especially for product **2**, this subjection is pronounced. The latter feature is known to be characteristic of chain processes [42]. Secondly, the H/D substitution drastically suppresses the formation of acetone, the key product of the free-radical substrate dehydration. Thus, we can firmly state that the pronounced KIE is observed for this product, reaching the value of 15.3±3.1 at the substrate concentration of 1 M.

Effects of the H/D substitution in the position C_2 of 2-propanol on its radiation-induced transformations in deaerated aqueous solutions

In our opinion, the observed KIE in the case of acetone formation from 1,2-propanediol/1,2-propanediol-2-d_1 cannot be fully explained by the different attitudes of initiators to the substrate. And this part aims to justify this point of view.

To assess the contribution of the initiation step to the observed KIE on the chain acetone formation (step 1 in Scheme (1)), a similar experiment with 2-propanol and 2-propanol-2-d_1 was carried out. The radiation chemistry of this substance is much simpler than that of 1,2-propanediol. The main product of radiolysis here is acetone, which is formed as a result of the disproportionation of prevailing $CH_3{\cdot}C(OH)CH_3$ radicals following the interaction of the substrate with ·H/·OH [12]. The radiation-chemical yields of acetone formation are presented in Table 2.

<**Table 2**>

In contrast to vicinal diol radicals, alcohol radicals such as $CH_3{\cdot}C(OH)CH_3$ do not form β-carbonyl radicals, which can accumulate as a result of a chain reaction with the substrate. The difference in G(acetone), equal to 1.2±0.2 times, indicates the negligible effect of the substrate reaction with radical water radiolysis products on the possible KIE. Although the calculated KIE value is 1.2, the 95% confidence interval from 1.0 to 1.4 does not allow us to claim an unambiguous KIE (despite P-value formally points to the difference in the G-values).

Effects of the H/D substitution in the positions $C_{2,3}$ of 2,3-butanediol on its radiation-induced transformations in deaerated aqueous solutions

The next issue to be addressed: is it crucial to have primary C-centered radicals after the radiation-induced dehydration of vicinal diols to observe a chain mechanism and pronounced KIE? To answer this, we conducted the radiation-chemical experiment with 2,3-butanediol and

2,3-butanediol-2,3-d_2, where secondary C-centered radicals are obtained during the course of the substrate transformations [12].

<**Fig. 2**>

The results obtained are presented in Fig. 2, from which it is evident that there is no KIE on the formation of 2-butanone, the product of interest.

Discussion

Radiolysis of pure water under the action of ^{60}Co γ-rays can be represented by the following scheme, which is also applicable in the case of dilute aqueous solutions (the primary G-values (μmol·J^{-1}) at pH of 4-9 are specified in the brackets [43]):

$$H_2O \xrightarrow{\gamma} e^-_{aq}\ (0.28),\ \cdot OH\ (0.29),\ \cdot H\ (0.06),\ H_2O_2\ (0.08),\ H_2\ (0.05),\ H^+_{aq}\ (0.36),\ OH^-_{aq}\ (0.06) \quad (3)$$

It should be noted that the molecular products (H_2O_2, H_2) practically do not interact with the solutes studied in the present work. On the contrary, the radical products (·OH, ·H, e^-_{aq}) are very active. So, ·OH radicals detach hydrogen atoms from an alcohol molecule with the rate constant of 10^8-10^9 M^{-1}·s^{-1}, and ·H – with the rate constant of 10^5-10^7 M^{-1}·s^{-1} [43], but hydrated electrons are inactive in relation to substrates that do not possess acceptor properties (alcohols, diols). If e^-_{aq} is not scavenged it can still interfere by reacting with the radicals or stable products formed from such substrates. But in dilute aqueous solutions with pH near 7 and in the absence of oxygen, e^-_{aq} can react primarily with H^+_{aq} ($k = 2.3 \cdot 10^{10}$ M^{-1}·s^{-1} [44]):

$$e^-_{aq} + H^+_{aq} \rightarrow \cdot H \quad (4)$$

and, in less degree, with H_2O molecules, which leads to the additional formation of hydrogen atoms.

Besides, the solvated electrons can interact with hydrogen peroxide (Equation (5), $k = 1{,}3 \cdot 10^{10}$ M^{-1}·s^{-1}, [43]) and carbonyl compounds, if the latter are present in sufficient concentrations in the solution.

$$e^-_{aq} + H_2O_2 \rightarrow \cdot OH + OH^-_{aq} \quad (5)$$

Thus, during the short-term radiolysis of aqueous, comparably diluted (≤ 1 M), deaerated solutions containing alcohols or diols, one can consider only the interactions of the substrate with ·OH and ·H radicals.

Schemes in Fig. 3 describe well-studied free-radical processes, which take place during the radiolysis of 1,2-propanediol in deaerated aqueous solutions. It has been reliably established by methods of pulse radiolysis, EPR, and with the use of spin traps and other acceptors, that radicals of vicinal diols (RC·(OH)-CH(OH)R') are unstable and they transform into the corresponding β-carbonyl radicals (RC(O)-·CH-R') with the simultaneous elimination of H_2O [12,45].

<**Fig. 3**>

In addition to radicals **C1** and **C2**, the formation of radical **C5** (·CH_2-CH(OH)-CH_2OH/·CH_2-CD(OH)-CH_2OH) is also possible, but, as shown by the method of EPR, it is minor compared to the former radicals [45,46], and converted into **C2** and **C1** by reacting with the substrate or undergoes β-fragmentation with the formation of **5** and ·CH_2OH. Although the reaction of recombination (dimerization) of **C1**–**C4** radicals cannot be excluded, the products formed during this process are also minor due to the low probability of the event when the radicals are in close proximity to each other (in other words, due to the low stationary concentration during radiolysis). It should be noted that the transformation of oxygen-centered radicals **O1** and **O2** into carbon-centered radicals **C1**–**C4** (via interaction with the substrate) is also observed. With the sufficient accumulation of carbonyl products, the processes of their interaction with e^-_{aq} should occur, but this is observed only under very long-term exposure to γ-rays.

1,2-Propanediol radiation-induced dehydration with the formation of acetone at an absorption dose rate of 0.110±0.003 Gy·s^{-1} in aqueous solutions is a non-branched chain process

The formation of a dehydration product in a straight (non-branched) chain reaction was shown by the example of indirect radiolysis of ethylene glycol deaerated aqueous solutions at a dose rate of *ca.* 0.64 Gy/s [43,47]. However, the values of G(dehydration product) characteristic of chain processes were not obtained at this dose rate in the case of 1,2-propanediol. At a dose rate of 0.64 Gy/s, G(acetone) in 0.1 M 1,2-propanediol solution (pH 6.0-6.5) did not exceed the sum of G_{OH} + G_H + G_e (taking into account the transformation of e^-_{aq} to ·H by reaction (4)) and was equal to 0.249-0.280 μmol·J^{-1} [12].

However, the radiation-chemical yields of acetone obtained in our (present) work for 0.1 and 1 M 1,2-propanediol solutions at pH 7.0±0.1, equal to 0.719±0.065 and 2.07±0.16 μmol·J^{-1} (Table

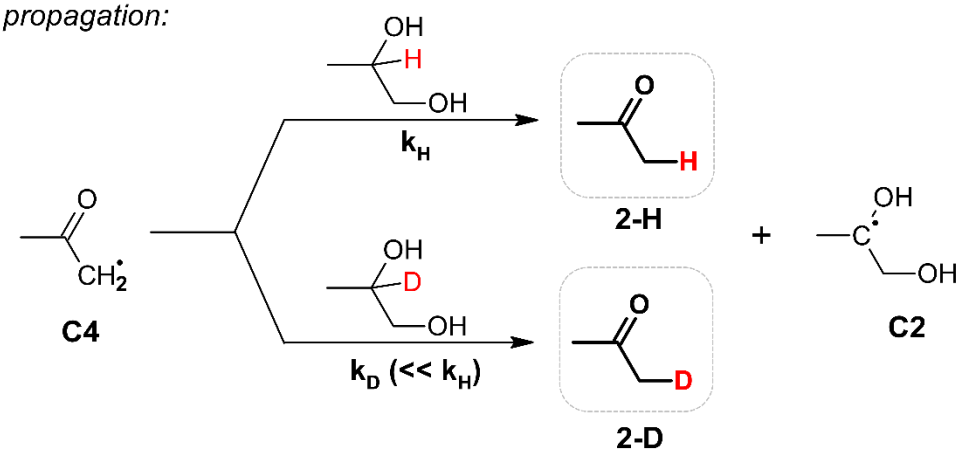
1), respectively, indicate the chain nature of acetone formation at the relatively low dose rate. In the work of Jiang and colleagues [48], the radiolysis of 1,2-propanediol solutions saturated with N_2O (to convert e^-_{aq} to ·OH) was studied at a dose rate of 0.1 $Gy \cdot s^{-1}$ (γ-rays of ^{60}Co), which is quite close to the value at which we conducted our radiation-chemical experiments. For the substrate concentrations of 0.2 and 2 M, the authors obtained G(acetone) equal to 1.55 and 3.05 $\mu mol \cdot J^{-1}$, respectively. Despite i) the unequal activity of the free radical system (in a system with N_2O there are much more ·OH radicals), ii) the concentration of substrates, iii) and some minor differences in the experimental conditions, we can say that the results of our work are consistent with the results of [48], which also demonstrate the chain formation of acetone. Indeed, G-values of radiolysis products may depend on the radiation dose rate inversely, i.e. increasing significantly with the decrease in dose rate [49].

Thus, the dose rate strongly affects G-values of the molecular products formed from vicinal diols. The point is that the increase in dose rate leads to a decrease in primary yields of water radiolysis radical products and, in contrast, the increase in primary yields water radiolysis molecular products. In other words, in diluted aqueous solutions the "radical-radical" recombination of corresponding water radiolysis products competes and may even prevail over the "radical-diluted compound" interactions [12,43]. For instance, it was shown on the example of deaerated 1.2 M ethylene glycol aqueous solutions that the total radiation-chemical yield of products decrease noticeably with increasing dose rate [12]. This dependence is particularly pronounced in the case of chain processes, as was shown for acetaldehyde formed from ethylene glycol at low dose rates [47], when the reaction rate depends on the concentration of initiators (·OH and ·H).

Deuteration of 1,2-propanediol in the α-C position relative to the secondary OH-group can quench its radiation-induced chain dehydration at an absorption dose rate of 0.110±0.003 $Gy \cdot s^{-1}$ in aqueous solutions

Once again, it is safe to postulate the chain mechanism of acetone radiation-induced formation from 1,2-propanediol. We suppose this effect is observed due to the different reactivity of the acetonyl radical **C4** in relation to the C–H/C–D bonds of the initial molecules. Thus, the introduction of deuterium into position C_2 of 1,2-propanediol slows the rate-limiting step of the acetone formation down.

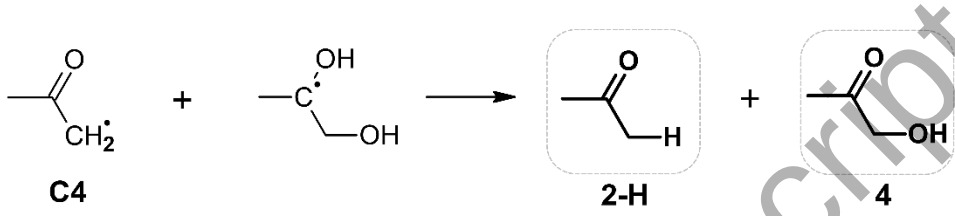
propagation:



(6)

(7)

termination:



(8)

From comparing the rate constants of the reactions of alcohols with ·OH, ·H, and carbon-centered radicals, it can be concluded that the latter will define the rate-limiting step in the acetone formation. One of the most active aliphatic C-centered radicals is $\cdot CH_3$. Although the rate of hydrogen atom transfer from an alcohol molecule also depends on the substrate structure, the following data [50] demonstrate that rate constants of such processes are much lower than those involving ·OH and ·H (~10^9 and 10^7 $M^{-1}\cdot s^{-1}$, correspondingly):

$$\cdot CH_3 + CH_3OH \rightarrow CH_4 + \cdot CH_2OH \ (k = 2\cdot 10^2\ M^{-1}\cdot s^{-1}) \quad (9)$$

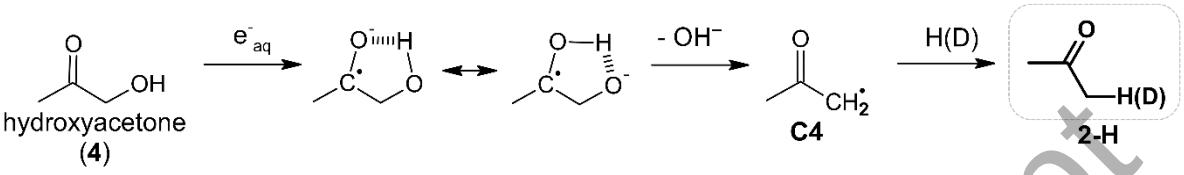
$$\cdot CH_3 + CH_3CH_2OH \rightarrow CH_4 + CH_3\cdot CHOH + \cdot CH_2CH_2OH \ (k = 6\cdot 10^2\ M^{-1}\cdot s^{-1}) \quad (10)$$

$$\cdot CH_3 + CH_3CH(OH)CH_3 \rightarrow CH_4 + CH_3\cdot C(OH)CH_3 + \cdot CH_2CH(OH)CH_3 \ (k = 3.4\cdot 10^3\ M^{-1}\cdot s^{-1}) \quad (11)$$

Taking into account the reduced reactivity of acetonyl and similar $\cdot CH_2C(O)R$ species (because of the resonance stabilization of acetonyl radical by the adjacent carbonyl group [51]), one can expect the rate constant of reaction (6) being not higher than ~10^3 $M^{-1}\cdot s^{-1}$. The conclusion is that reaction (6) ((7)) is the rate-limiting step of the acetone formation process studied. And the significant KIE is related to this interaction. In addition, the H/D substitution blocks another secondary way of **C2** formation, which may also affect G_H(acetone)/G_D(acetone): the transformation of **C3** to **1** through interaction with the substrate. In the case of 1,2-propanediol-d_1, the formation of **C1** is predominant here, whereas the non-substituted substrate gives rise to both **C1** and **C2** radicals in this reaction, with **C2** being prevalent. The fact that **C3** abstracts H in

the tertiary position more actively than in the secondary one also leads to a "leak" of the radical-propagator from the hypothetical chain, which might describe the formation of **1**. This is one of the reasons why we do not observe G(propanal) characteristic for a chain process, whereas G(acetone) reaches the value of 2.07±0.16 μmol·J^{-1} at c(1,2-propanediol) = 1 M.

The next quite significant point concerning the results of the experiment is the influence of the hydroxyacetone impurity in the deuterated substrate on G(products). Hydroxyacetone contributes to the formation of acetone by reacting with e^-_{aq}:



(12)

This leads to the overestimation of G(acetone) in 1,2-propanediol-2-d_1 samples. In fact, in the presence of this carbonyl impurity, the strong KIE of acetone formation is observed, and in the case of ultimately pure 1,2-propanediol-2-d_1 one should expect even a greater G_H(acetone)/G_D(acetone) value.

Alcohols that are not characterized by chain radiation-chemical transformations, do not show a significant KIE when deuterated in the α-C position relative to the OH-group

One can expect that not only reactions (6) and (7) but also the abstraction of H or D by the radical water radiolysis species from 1,2-propanediol or 1,2-propanediol-2-d_1, respectively, are the steps sensitive to isotope substitution.

Concerning the radical water radiolysis species, we must exclude from consideration ·OH-radicals. These well-known " furious killers" in all free-radical reactions are too active to differentiate energetically hydrogen and deuterium atom transfer processes. At the same time, hydrogen atoms (·H) are less reactive than ·OH radicals, since H–H bond formation is less exothermic than that of H–OH, and therefore ·H are likely more selective. Indeed, this selectivity was shown in some works devoted to H/D abstraction by ·H from 2-propanol/2-propanol-d_7 in aqueous solutions [52–54]. However, the results of our research (Table 2) show that when both ·H and ·OH radicals are present in the solution, no KIE on the formation of acetone from 2-propanol and 2-propanol-2-d_1 is observed. This finding rejects the idea that the interaction of the radical water radiolysis species with the C–H/C–D bonds plays a key role in the manifestation of the KIE related to the formation of carbonyl products in (1). Therefore, we assume the carbon-

centered acetonyl radical is the one that differentiates C–H/C–D bonds in 1,2-propanediol/1,2-propanediol-2-d_1 molecules.

Propagating the chain process of carbonyl product formation is characteristic only for the primary carbon-centered radicals derived from vicinal diols

In the systems studied, ·OH and ·H radicals react with 2,3-butanediol/2,3-butanediol-2,3-d_2 molecules, forming mainly radicals **C6** (Fig. 4). Radicals **C6** are involved in two processes: disproportionation (formation of 3-hydroxybutanone (acetoin), product **8**), and dehydration (formation of 2-oxobutyl radicals **C7**). 2-Butanone (methyl ethyl ketone, product **7**) is obtained by reactions of **C7** with either the initial substrate molecules (**C6** are also regenerated) or **C6** radicals (disproportionation that yields product **8** as well). Besides, a certain quantity of C-C destruction product **9** is observed in the system after irradiation.

<**Fig. 4**>

The observed "ordinary" values of radiation-chemical yields of 2-butanone formation from 2,3-butanediol and 2,3-butanediol-2,3-d_2 and their statistical similarity are explained as follows. **C7** is additionally stabilized by the adjacent methyl group compared to **C4**, thus less reactive towards H(D) abstraction from the corresponding substrate molecule. So, one should expect less contribution of the chain process, which is analogous to that of 1,2-propanediol conversion to acetone, to the reduction of **C7** to **7**. G(2-butanone) obtained at a dose rate of 0.110±0.003 in the present study is consistent with the value of 0.126-0.162 μmol·J^{-1} found in earlier works at 0.64 Gy·s^{-1} [12]). Thus, the independence of G(2-butanone) from the dose rate may in some way indicate a low contribution or even the absence of the chain process in the formation of **7** from 2,3-butanediol.

To sum up, when switching from primary to secondary radicals in (1), the probability of the chain formation of the corresponding carbonyl product is reduced sharply, and the isotopic substitution does not affect free radical dehydration of vicinal diols of the $R_1CH(OH)CH(OH)R_2$ (R_1, $R_2 \neq H$) type.

Conclusion

The studies shown here clearly indicate the chain nature of the radiation-induced formation of acetone in 1,2-propanediol deaerated aqueous solutions and quenching the process by replacing hydrogen with deuterium in position C_2 of the initial compound. This is due to the formation of acetonyl radicals, which propagate the chain mechanism and abstract hydrogen atoms from the substrate molecule more likely than deuterium atoms. Propanal, the second product of 1,2-propanediol dehydration, is characterized by lower G-values of its formation since the precursor $CH_3{\cdot}CHCHO$ radicals are less active and do not participate in the corresponding chain propagation reaction. The exceptional role of primary β-carbonyl radicals in the radiation-induced chain dehydration of diols and, as a result, in the appearance of a strong KIE, is also demonstrated by the absence of a KIE when 2-butanone is formed from 2,3-butanediol or 2,3-butanediol-2,3-d_2 in deaerated aqueous solutions.

Some general considerations can be drawn from the observed effects, which affords a deeper insight into the reactivity of hydroxyl-containing compounds with radical species. By substituting H with D in the positions geminal to OH and functional groups we can expect the suppression of free radical fragmentation in compounds prone to the radiation-induced 2β-cleavage followed by the formation of corresponding primary β-carbonyl radicals. Examples of such compounds are mono- and diglycerides (but not triglycerides), lysophospholipids, phosphatidylglycerol. For similar substances, but capable of forming only secondary β-carbonyl radicals (carbohydrates, glycolipids, ceramides, sphingomyelins, ribonucleotides) there is no reason to assume the same.

Disclosure of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Palina Nepachalovich: Investigation, Visualization, Formal analysis, Writing – Original draft preparation and Editing; **Oleg Shadyro:** Supervision, Conceptualization, Writing – Review; **Andrei Bekish:** Resources (synthesis of deuterated substrates), Conceptualization. **Vadim Shmanai:** Conceptualization.

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Figure legends

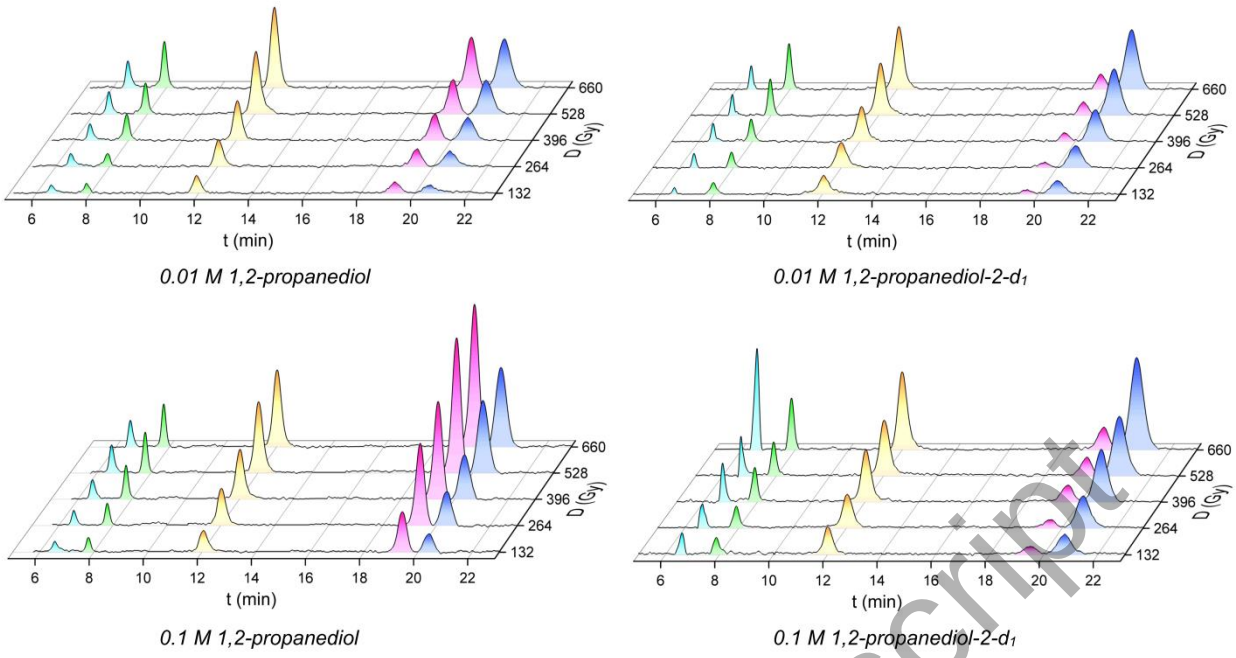


Fig. 1. LC-UV analyses of irradiated 0.01 M and 0.1 M solutions of 1,2-propanediol and 1,2-propanediol-2-d_1 at various doses (dose rate = 0.110±0.003 $Gy \cdot s^{-1}$) after the derivatization of the carbonyl compounds with DNPH. Peaks from left to right: hydroxyacetone (turquoise), formaldehyde (green), acetaldehyde (yellow), acetone (purple), propanal (blue). Each chromatogram presented is the result of subtracting the corresponding control chromatogram (a non-irradiated sample within the set, 0 Gy) from the original chromatogram of a given dose. The aim of this pre-processing is to visualize the radiolytic accumulation of products only, where any interference coming from the derivatization step is excluded.

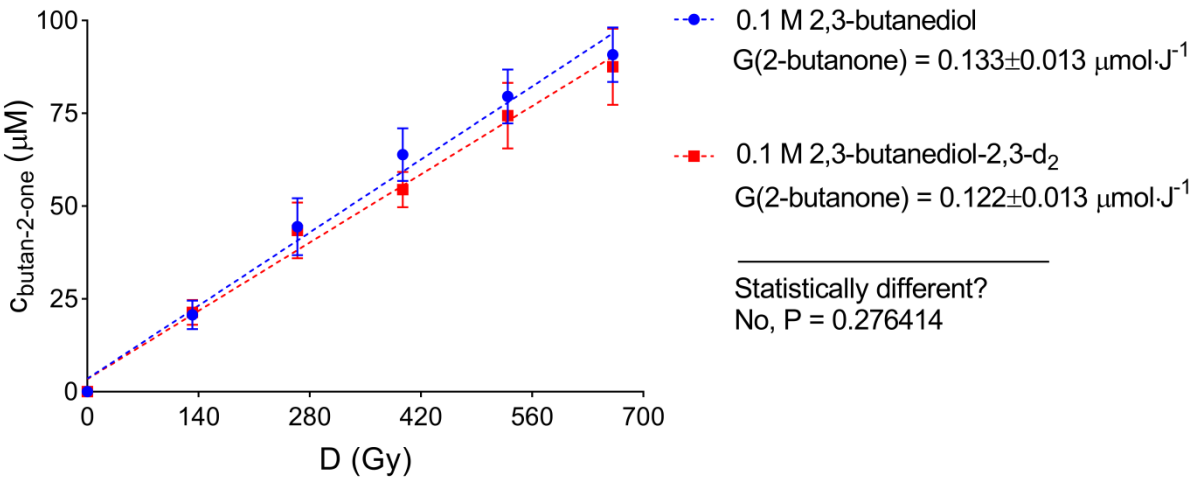


Fig. 2. Accumulation curves for 2-butanone formed during the radiolysis of 2,3-butanediol and its deuterated analogue solutions in 50 mM phosphate buffer (pH 7.0±0.1). Dose rate = 0.110±0.003 Gy·s^{-1}. Radiation-chemical yields of 2-butanone and the t-test result are presented on the right (*p = 0.95*).

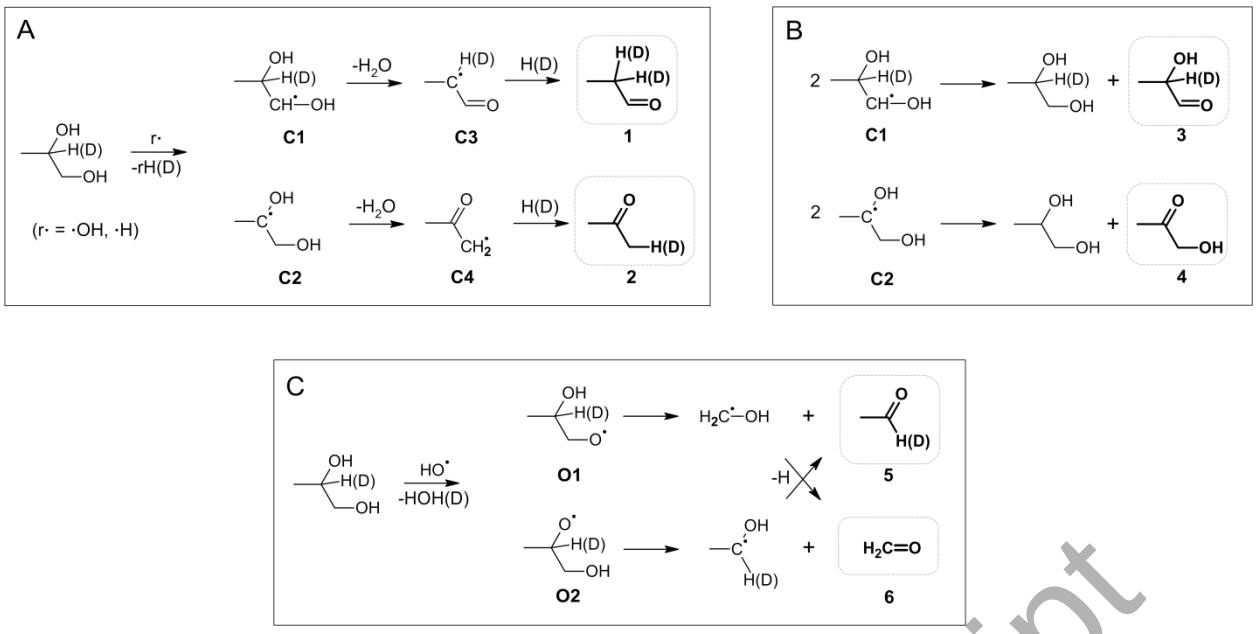


Fig. 3. Schemes describing the indirect radiolysis of 1,2-propanediol and its deuterated analogue in a deaerated aqueous solution. A: formation and dehydration of major carbon-centered radicals; B: disproportionation of major carbon-centered radicals; C: formation and destruction of major oxygen-centered radicals.

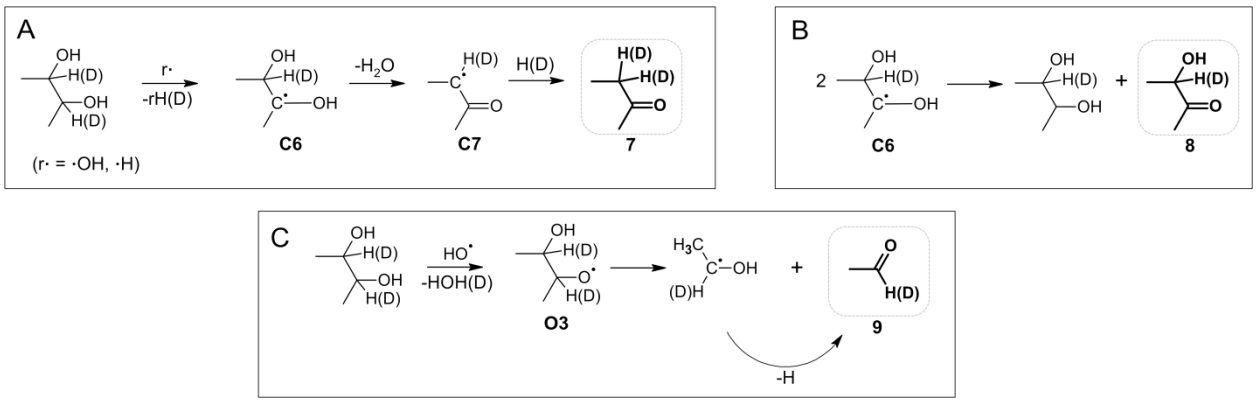
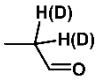
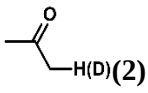
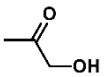


Fig. 4. Schemes describing the indirect radiolysis of 2,3-butanediol and its deuterated analogue in a deaerated aqueous solution. A: formation and dehydration of a major carbon-centered radical; B: disproportionation of the major carbon-centered radical; C: formation and destruction of an oxygen-centered radical.

Table 1. G-values (μmol·J^{-1}) of the products formed during the radiolysis of 1,2-propanediol and its deuterated analogue solutions in 50 mM phosphate buffer (pH 7.0±0.1). Dose rate = 0.110±0.003 Gy·s^{-1}.

c (substrate), M	**0.01**		**0.1**		**1**	
Product \ Substrate	1,2-propanediol	1,2-propanediol-2-d_1	1,2-propanediol	1,2-propanediol-2-d_1	1,2-propanediol	1,2-propanediol-2-d_1
 **(1)**	0.050±0.008	0.056±0.007	0.094±0.011	0.093±0.011	0.183±0.027	0.131±0.020
Statistically different?	No, P = 0.469439		No, P = 0.923059		Yes, P = 0.013525 1.4±0.4 times	
 **(2)**	0.170±0.018	0.041±0.005	0.719±0.065	0.081±0.008	2.07±0.16	0.135±0.017
Statistically different?	Yes, P<0.000001 4.10±1.0 times		Yes, P<0.000001 8.9±1.7 times		Yes, P<0.000001 15.3±3.1 times	
 **(4)**	0.097±0.029	–*	0.094±0.037	–*	0.145±0.015	–*
	–		–		–	
 **(5)**	0.044±0.005	0.040±0.014	0.052±0.005	0.056±0.007	0.071±0.008	0.048±0.008
Statistically different?	No, P = 0.619761		No, P = 0.290156		Yes, P = 0.000831 1.5±0.4 times	
 **(6)**	0.028±0.011	0.025±0.005	0.030±0.004	0.035±0.011	0.040±0.007	-
Statistically different?	No, P = 0.687394		No, P = 0.428283		-	

p = 0.95

***Hydroxyacetone was not quantified due to its presence as an impurity in the deuterated substrate*

Table 2. G-values (μmol·J^{-1}) of acetone formed during the radiolysis of 2-propanol and its deuterated analogue solutions in 50 mM phosphate buffer (pH 7.0±0.1). Dose rate = 0.110±0.003 Gy·s^{-1}.

c (substrate), M	**0.1**	
Product \ Substrate	2-propanol	2-propanol-2-d_1
	0.177±0.015	0.145±0.015
Statistically different?	Yes, P = 0.002025, 1.2±0.2 times	

p = 0.95